REMARKS

Claims 28, 29, 31, 32, 34, 36-47, 55, 57, 58, and 62-66 are pending and have been examined. Claims 28, 29, 31, 32, 34, 36-47, 55, 57, 58, and 62-66 stand rejected. Claims 28, 31, and 63 have been amended. Claims 29, 32, 34, 36-47, 55, 57, 58, 62, and 64-66 have been canceled without acquiescing to the Examiner's arguments and without prejudice to applicant's right to pursue the subject matter of these claims in one or more subsequent applications. Applicant respectfully requests reconsideration and allowance of Claims 28, 31, and 63.

Rejection of Claims Under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected Claim 44 under 35 U.S.C. § 112, second paragraph, as being indefinite. Claim 44 has been canceled. Accordingly, this ground for rejection is now moot.

Rejection of Claims Under 35 U.S.C. § 112, First Paragraph (Enablement)

The Examiner has rejected Claims 28, 29, 31, 32, 34, 36-47, 55, 57, 58, and 62-66 under 35 U.S.C. § 112, second paragraph, as not being enabled by the specification. As a preliminary matter, applicant notes that the subject matter relating to $p21^{Cip1}$ and $p57^{Kip2}$ has been canceled from the claims. The pending claims are now directed to the use of antisense molecules directed to mammalian $p27^{Kip1}$ to treat hearing loss. Accordingly, the Examiner's rejections as they apply to $p21^{Cip1}$ and $p57^{Kip2}$ and inhibitors other than $p27^{Kip1}$ antisense molecules are moot. In addition, Claims 29, 32, 34, 36-47, 55, 57, 58, 62, and 64-66 have been canceled.

According to the Examiner, the experiments in the specification only demonstrate proliferation in response to inhibition of $p27^{Kip1}$ but do not show that the resulting cells are properly developed and provide viable sensory cells that result in a treatment for hearing loss such as perception deafness. Applicant respectfully disagrees. The specification states that cell division represents the decisive step in the hair cell regeneration process (specification, page 9, line 2). Moreover, the specification notes that the mitosis of the supporting cells in $p27^{Kip1}$

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knockout mice results in mature sensory cells and that these knockout mice consequently have more hair cells than normal mice (specification, page 9, lines 6-9). Therefore, as pointed out in the specification, the experiments enable the conclusion to be drawn that in addition to cell division, "there is also differentiation or maturation to hair sensory cells and finally a functional recovery of auditory function of the sensory organ" (specification, page 9, lines 1-5).

The specification further describes that regeneration of sensory cells is possible even when $p27^{Kip1}$ activity is not completely eliminated because a gene dose-dependent effect on the regeneration of the sensory cells is observed in $p27^{Kip1}$ heterozygous mice (specification, page 9, lines 25-28). Thus, after destroying the hair cells by the systemic administration of the ototoxic antibiotic amikacin to $p27^{Kip1}$ heterozygous mice, examination of the cochlea revealed regenerated hair cells (specification, page 9, lines 10-18). Furthermore, appended hereto as Attachment A is the Second Declaration of Dr. Jonathan Kil ("the Second Kil Declaration"), which describes experiments carried out by Dr. Kil and his co-workers to assess the proliferation of supporting cells in the organ of Corti and the level of hair cell regeneration in response to aminoglycoside ototoxicity in $p27^{Kip1}$ homozygous mutant mice. Specifically, administration of amikacin to $p27^{Kip1}$ homozygous mutant mice resulted in increases in proliferation of many of the supporting cell types in the organ of Corti as well as hair cell regeneration (Second Kil Declaration, paragraphs 4-6, Table 1). In addition, the Second Kil Declaration describes experiments demonstrating that partial elimination of $p27^{Kip1}$ function in $p27^{Kip1}$ heterozygous mice results in an improvement in auditory function after treatment of the mice with amikacin (Second Kil Declaration, paragraph 7, Table 2, and Figure 1). Moreover, as acknowledged by the Examiner, the Declaration of Dr. Jonathan Kil appended to the amendment filed on March 7, 2003 ("the First Kil Declaration"), demonstrates that the local administration of $p27^{Kip1}$ antisense molecules, which result in a dose-dependant reduction of $p27^{Kip1}$ mRNA and protein

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levels, causes proliferation of cells in the organ of Corti. In combination, the results described in the First Kil Declaration and the Second Kil Declaration show that reducing $p27^{Kip1}$ activity by locally administering $p27^{Kip1}$ antisense molecules results in proliferation of cells in the organ of Corti and that the partial elimination of $p27^{Kip1}$ function in $p27^{Kip1}$ heterozygous mice results in an improvement in auditory function after treatment of the mice with amikacin.

The Examiner further states that the specification does not provide specific guidance on the treatment of perception deafness because perceptive deafness encompasses a broad range of diseases and disorders, including, for example, deafness caused by destruction of sensory cells wherein the origin of the destruction is genetic, unrelated to cell cycle inhibitors. Applicant respectfully disagrees because the methods of the invention are not limited to a particular cause for the destruction of sensory cells that leads to hearing loss. Specifically, the methods of the invention are not directed to reversing the destruction of sensory cells but rather to stimulating the proliferation of the *supporting cells* present in the sensory epithelium, thereby promoting the regeneration of functional sensory cells (specification, page 3, lines 12-22). As described in the specification, necrotic sensory cells can be replaced in birds by division of a supporting cell and subsequent maturation to form a new supporting cell and a sensory hair cell (specification, page 1, lines 30-36). This process does not occur naturally in mammals. One theory that accounts for the difference between birds and humans is that in the bird cochlea, supporting cells only temporarily leave the cell cycle, whereas supporting cells in mammals irreversibly leave the cell cycle (specification, page 2, lines 15-21). The specific hypothesis addressed by the present invention is that mammalian supporting cells are prevented from reentering the cell cycle by the presence of a cell cycle inhibitor such as $p27^{Kip1}$. Applicant has shown that by inhibiting the activity of the cell cycle inhibitor $p27^{Kip1}$ the supporting cells in mammalian cochlea reenter the cell cycle and their progeny can differentiate into new sensory hair cells. Accordingly, the

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manner in which the sensory cells were destroyed to produce hearing loss is not critical provided that there are viable supporting cells that can proliferate in response to the inhibition of $p27^{Kip1}$ activity.

This point is corroborated by a recent review of biological approaches to preventing and treating hearing loss (Holley (2002) *Br. Med. J.* 63:157-169, appended as Attachment C). This review clarifies that the cause of over 80% of cases of hearing loss is related to degeneration and death of sensory hair cells and their associated spiral ganglion neuron (Holley (2002) *Br. Med. J.* 63:157-169, abstract, page 157, first paragraph). This review notes that in some vertebrates, such as birds, sensory hair cells can be replaced by division and differentiation of supporting cells (Holley (2002) *Br. Med. J.* 63:157-169, page 162, second paragraph). Moreover, this review states that in mammalian epithelia, "[i]t may be possible to induce proliferative replacement of hair cells by suppressing p27 or associated proteins in supporting cells." Notably, the review does not distinguish between replacing hair cells in cases where the cause of hearing loss is related to cell cycle inhibitors or unrelated to such cell cycle inhibitors. Applicant submits that the method of the invention induces such a proliferative replacement of hair cells by inhibiting the activity of $p27^{Kip1}$. As described above, the First Kil Declaration shows that reducing $p27^{Kip1}$ activity by locally administering $p27^{Kip1}$ antisense molecules results in proliferation of cells in the organ of Corti in Guinea pigs and in cochlear cultures.

For the reasons described above, applicant submits that the specification provides an enabling description for Claims 28, 31, and 63. Withdrawal of this ground of rejection is respectfully requested.

Rejection of Claims Under 35 U.S.C. § 112, First Paragraph (Written Description)

The Examiner has rejected Claims 28, 29, 31, 32, 34, 36-47, 55, 57, 58, and 62-66 under 35 U.S.C. § 112, second paragraph, as lacking an adequate written description. As described in

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the preceding section, the subject matter relating to $p21^{Cip1}$ and $p57^{Kip2}$ has been canceled from the claims. The pending claims are now directed to the use of antisense molecules directed to mammalian $p27^{Kip1}$. Accordingly, the Examiner's rejections as they apply to $p21^{Cip1}$ and $p57^{Kip2}$ and inhibitors other than antisense molecules to mammalian $p27^{Kip1}$ are moot. In addition, Claims 29, 32, 34, 36-47, 55, 57, 58, 62, and 64-66 have been canceled.

Applicant respectfully submits that Claims 28, 31, and 63 are supported by an adequate written description. The sequences of mammalian $p27^{Kip1}$ genes are highly related. For example, the predicted amino acid sequences of human, mouse, and mink $p27^{Kip1}$ proteins are about 90% identical (see Polyak et al. (1994) *Cell* 78:59-66, already of record, page 61, last line, to page 62, line 2). A Clustal W alignment of the nucleic acid sequences coding for these proteins (Accession numbers GI:48146914, GI:516545, and GI:516543, respectively) shows that they are more than 85% identical (Clustal W alignment, appended as Attachment D). As noted by the Examiner, Coats et al. (1996) *Science* 272:877-880 and Hauser et al. (1997) *Cell Growth and Differentiation* 8:203-211, already of record, disclose antisense inhibitors for $p27^{Kip1}$. These antisense inhibitors of $p27^{Kip1}$ were known and used prior to the filing date of the present application. Significantly, one of the antisense inhibitors used in Coats et al. and in Hauser et al. (antisense to base pairs 306 to 320 of murine $p27^{Kip1}$, Coats et al. (1996) *Science* 272:877-880, page 880, footnote 14) is directed to a region that is identical in the mouse, human, and mink genes. Moreover, treatment with this same antisense inhibitor results in a dose-dependant reduction of $p27^{Kip1}$ mRNA and protein levels and causes proliferation of cells in the organ of Corti in Guinea pigs (see First Kil Declaration). Furthermore, another antisense inhibitor that has been effectively used to reduce $p27^{Kip1}$ in Guinea pigs is directed to another region that is almost identical in mouse, human, and mink $p27^{Kip1}$ (see First Kil Declaration, SPI5505, antisense to base pairs 421 to 445; Clustal W alignment). Therefore, one of skill in the art would

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recognize that the inventor possessed the invention of Claims 28, 31, and 63. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Rejection of Claim Under 35 U.S.C. § 102(b)

The Examiner has rejected Claim 55 under 35 U.S.C. § 102(b) as being anticipated by Hauser et al., *Cell Growth and Differentiation* 8:203-11, 1997. Claim 55 has been canceled. Accordingly, this ground of rejection is now moot.

CONCLUSION

In view of the foregoing claim amendments and remarks, applicant respectfully submits that Claims 28, 31, and 63 are in condition for allowance. Reconsideration and favorable action are requested.

Respectfully submitted,

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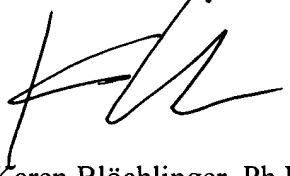

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